Chapter 3 Water Quality

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Introduction:

An integral component of any study to evaluate the overall quality of estuarine habitats and the resources supported by those habitats is an assessment of the physical, chemical, and biological conditions of the water column. Due to the closure of more than 46,000 acres of shellfish harvesting grounds in Beaufort County, a major focus of the water column biota investigation included a characterization of the potential origin, human versus animal, of the fecal coliform bacteria responsible for these closures.

The waters of the State are classified in regulation to establish resource protection goals for each water body (SCDHEC, 1998a). Water bodies classified as Outstanding Resource Waters (ORW) or Shellfish Harvesting Waters (SFH) receive the highest level of protection.

The entire Okatee River from its headwaters to the confluence with the Colleton River is classified as ORW. Class ORW waters are freshwaters or saltwaters which constitute an outstanding recreational or ecological resource, or those freshwaters suitable as a source for drinking water supply purposes, with treatment levels specified by the Department. No discharges from domestic, industrial, or agricultural waste treatment facilities are allowed to Class ORW waters. Open water dredged spoil disposal areas are also prohibited in Class ORW waters. Because of these prohibitions, Class ORW waters should exhibit conditions that represent a natural state. Specific numeric Standards are those of the water body's classification prior to its reclassification to ORW. In the case of the Okatee River this is Class SFH.

Broad Creek from its headwaters to Calibogue Sound is classified as shellfish harvesting waters (Class SFH). Class SFH waters are tidal saltwaters protected for shellfish harvesting, and are also suitable for primary and secondary contact recreation, crabbing, fishing and the survival and reproduction of a balanced indigenous aquatic community. There are very specific numeric Standards for Class SFH waters to guarantee that these uses are protected in spite of any discharges that may be present.

Methods:

SCDHEC Water Quality Sampling

SCDHEC water quality samples were collected one time at 15 sites in each drainage basin during August 1997. Six sites were located in tidal creeks, six were in subtidal river areas of the larger drainage system, and three were located on intertidal flats of mainstem river system (Figures 3.1 and 3.2). The tidal creek and subtidal river sites were randomly located within each of the six subzones established along the length of each drainage system (see Chapter 2). The intertidal river sites were also randomly selected from the three larger zones representing the upper (headwater), middle and lower (seaward) portions of each drainage system. As stated previously, the primary objective of this study was to provide a better understanding of existing conditions in different habitats of each drainage system using an unbiased sampling design. The study was not designed to target specific activities, such as evaluating the effects of marinas, boating activities, urban runoff, etc. Rather, it was designed to evaluate the integrated effects of all activities that may affect the quality of these water bodies. All station positions were located using differentially-corrected Geographic Positioning Systems (GPS). Sampling in each of the tidal creeks took place near the downstream end of the upper (landward) 300 m section. All water quality samples were collected at or near half tide on an ebbing tide.

The Okatee River was sampled on August 19, 1997, and Broad Creek was sampled on August 20, 1997. This sampling period was characterized by exceptional spring tides with extreme high and low tides. There was a 1.3 inch rainfall the night of August 19, preceding the sampling of Broad Creek. The runoff from this storm coupled with the extreme high tides that inundated more land area closer to upland sources than normal appears to have influenced some of the water quality results for Broad Creek. The Okatee River samples were collected after a period of relatively dry weather.

Instantaneous dissolved oxygen (DO, mg/l), pH (SU), specific conductance (mS/cm), salinity (ppt), and water temperature (°C) were measured in situ at a depth of 0.3 meters, representing surface conditions, following standard SCDHEC procedures (SCDHEC, 1997). DO, specific conductance, salinity and water temperature were also measured in situ at the bottom and mid-depth at sites greater than one meter deep. Percent dissolved oxygen saturation (%DO Sat) was calculated from in situ DO, temperature, and salinity data. Surface samples were collected for analysis of five-day biochemical oxygen demand (BOD₅, mg/l), turbidity (NTU), alkalinity (mg/l), total suspended solids (mg/l), ammonia nitrogen (NH₃+NH₄, mg/l), total Kjeldahl nitrogen (mg/l), nitrate nitrogen (NO₂+NO₃, mg/l), total phosphorus (mg/l), total organic carbon (mg/l), chlorophyll-a (ug/l), and fecal coliform bacteria (# colonies/100 ml). All samples were collected, preserved and transported following standard SCDHEC procedures (SCDHEC, 1997). All analyses were conducted following standard SCDHEC procedures (SCDHEC, 1981, 1994). The results of all analyses are presented in Appendix 3.1, and selected results are summarized in Table 3.1. The positive fecal coliform enumeration media were transferred to the NOS laboratory for Escherichia coli typing.

We did not attempt to analyze all possible contaminants in the water column because of their transient nature, being associated more with runoff than continuous sources. Rather, heavy metals and organic compounds were analyzed in the sediments (see Chapter 4) and in the oyster tissue (see Chapter 5) where they would be more likely to accumulate and therefore presented the most likely media for detection.

Throughout this chapter, where measured parameters have no State Standards, values are compared to statistics compiled from other SCDHEC saltwater monitoring data collected from 1993 through 1997 (SCDHEC, 1998b). Terminology used in these comparisons includes an indication of high concentrations, which represent a value greater than 90% of the values measured in other SCDHEC saltwater monitoring data, and an indication of very high concentrations, representing values greater than 95% of the values measured in other SCDHEC saltwater monitoring data (Table 3.2).

SCDNR Hydrolab Deployments

Salinity (ppt), conductivity (mS/cm), pH (SU), temperature (°C), water depth (m), and either dissolved oxygen (DO, mg/l) or percent dissolved oxygen saturation (%DO Sat, percent) or both, depending on the probe, were recorded electronically with a "Datasonde 3" (DS3) multiprobe data logger manufactured by Hydrolab Corporation. Where DO only was measured, %DO Sat was calculated using salinity and temperature (Okatee River sites T-2 and T-5). Conversely, where %DO Sat only was measured, DO was calculated (Okatee River sites R-1, R-2, R-5, R-6 and T-1). Continuous measurements of these parameters were made from a single near-bottom depth at 30-min intervals over approximately a 25-hr period to complete a full set of tidal cycles at six sites located in tidal creeks and six sites in subtidal river areas of the larger drainage system (Figures 3.1 and 3.2). Summaries of all Hydrolab measurements are presented in Appendix 3.2, and selected summary results are summarized in Table 3.3. All raw hydrolab measurements are presented in Appendix 3.3.

In Broad Creek the probes went dry for short periods at tidal creek sites T-1 through T-4. In the Okatee River, the hydrolab record was interrupted at sites T-1, T-3 and to a lesser extent at T-6. Rainfall was reported in the Broad Creek area during the deployment at site R-3. Although there were no rain records available, Okatee River sites T-3 and R-3 were deployed on the same date, and likely were also influenced by rainfall.

Findings:

Dissolved Oxygen:

Oxygen is essential for the survival and propagation of aquatic organisms. If the amount of oxygen dissolved in water falls below the minimum requirements for survival or reproduction, aquatic organisms or their eggs and larvae may die. Dissolved oxygen (DO) varies greatly due to natural phenomena, resulting in daily and seasonal cycles. Different forms of pollution also can cause declines in DO.

Changes in DO levels can result from temperature changes or the activity of plants and other organisms present in a water body. The natural diurnal (daily) cycle of DO concentration is well documented. Dissolved oxygen concentrations are generally

lowest in the morning, climbing throughout the day due to the release of oxygen through photosynthesis by aquatic plants and peaking near dusk, then steadily declining during the hours of darkness as it is depleted by plant and animal respiration.

There is also a seasonal DO cycle in which concentrations are greater in the colder, winter months and lower in the warmer, summer months. Flushing, reaeration, and the extent of saltwater intrusion all affect dissolved oxygen values.

Aquatic populations exposed to anoxia (DO<0.3 mg/l) or severe hypoxia (DO<2.0 mg/l) for even brief periods can experience significant mortality. In short exposure laboratory experiments, DO concentrations less than 0.21 mg/l have been shown to be lethal to several benthic invertebrates (Theede, 1973). Long-term chronic effects on survival can result from prolonged exposure to less severe hypoxia.

The State Standard for dissolved oxygen in Broad Creek and the Okatee River is a daily average not less than 5.0 mg/l, with a low of 4.0 mg/l. Compliance with this Standard is generally assessed near the surface. The vast majority of DO data collected during this study were near the bottom, but the observed bottom data can be compared to the Standard for strictly informational purposes. Other research projects in Southeastern estuaries have developed regional near-bottom DO guidelines that were also used to evaluate the data from this study. For the Carolinian Province Environmental Monitoring and Assessment Program - Estuaries (EMAP-E), Hyland et al. (1998) classified a site as "degraded" based on DO if near-bottom dissolved oxygen was less than 0.3 mg/l at any time, less than 2.0 mg/l for 20% or more of the observations, or less than 5.0 mg/l for all observations over a 24-hr time series. Holland et al. (1996) also use the occurrence of %DO saturation of <28% in 20% or more of the observations as an indication of degraded conditions.

Broad Creek:

SCDHEC Instantaneous Measurements:

Surface measurements: One intertidal river site (I-4) had a DO <5.0 mg/l, five tidal creek sites (T-1, T-3, T-4, T-5, T-6) had DO <5.0 mg/l, with three of those also <4.0 mg/l (T-1, T-4, T-6; Appendix 3.1, Table 3.1). All subtidal river sites had DO concentrations greater than 5.0 mg/l.

Bottom measurements: Where bottom measurements were taken (I-6, R-2 through R-6, T-3 and T-6), the conclusions are identical to the surface measurements, suggesting a well mixed water column.

Continuous Bottom Measurements:

Mainstem Sites: A daily mean DO less than 5.0 mg/l was observed at five sites (R-1 through R-5), with values less than 4.0 mg/l occurring at all of those sites as well (Figure 3.3 and 3.4, Appendices 3.2 and 3.3, Table 3.3). At the lower site (R-6) mean

DO was >5.0 mg/l with no values less than 4.0 mg/l. At the most upstream site (R-1) there were four DO values less than 2.0 mg/l (8.3%) and three %DO saturation values less than 28 (6.25%). In general, DO and %DO saturation variance and range decreased from upstream to downstream. None of the sites were classed as "degraded" using the EMAP criteria.

Tidal Creek Sites: The daily mean DO was less than 5.0 mg/l at all six sites, with concentrations less than 4.0 mg/l occurring at all sites as well (Figure 3.3 and 3.4, Appendices 3.2 and 3.3, Table 3.3). T-1 had seven values less than 2.0 mg/l (17.5% of samples) and five %DO saturation values less than 28% (12.5% of the samples). T-4 had one DO value less than 2.0 mg/l, and all values were less than 5.0 mg/l, qualifying as "degraded" under the EMAP protocol. T-5 had 10 values less than 2.0 mg/l with %DO saturation less than 28% (19.6% of samples), and the minimum value was 0.08 mg/l. Therefore, this site also classed as "degraded" under the EMAP criteria.

In general, the variance in DO and %DO saturation was higher in the tidal creeks than in the mainstem (Appendix 3.2). Average %DO saturation was lower in the tidal creeks than in the mainstem.

Okatee River

SCDHEC Instantaneous Measurements:

Surface measurements: The DO at two of the intertidal river sites (I-2 and I-6) was less than 5.0 mg/l, and I-6 was also less than 4.0 mg/l (Appendix 3.1, Table 3.1). Two of the subtidal river sites, R-5 and R-6 both exhibited a DO of less than 4.0 mg/l. DO was less than 5.0 mg/l at two tidal creek sites (T-2 and T-6).

Bottom measurements: Where bottom measurements were taken (I-2, I-6, R-1 through R-6, T-4 and T-6), the findings are identical to the surface measurements, suggesting a well mixed water column.

Continuous Bottom Measurements:

Mainstem Sites: Only the two middle zone sites (R-3 and R-4) had a daily average DO less than 5.0 mg/l, and both had values less than 4.0 mg/l (Figure 3.3 and 3.4, appendices 3.2 and 3.3, Table 3.3). Values less than 4.0 mg/l also occurred at R-1. No DO concentrations less than 2.0 mg/l and no %DO saturation values less than 28% were measured. None of the sites would be classed as "degraded" using the EMAP protocol. Percent DO saturation and maximum DO concentrations were generally higher in the mainstem of the Okatee River than in Broad Creek.

Tidal Creek Sites: Mean daily DO was less than 5.0 mg/l at five out of six sites (T-1 through T-4, and T-6), with values less than 4.0 mg/l occurring at all six sites (Figure 3.3 and 3.4, Appendices 3.2 and 3.3, Table 3.3). Site T-2 had one DO value less than 2.0 mg/l and one %DO saturation less than 28%. None of the sites would be classed

as "degraded" using the EMAP protocol. In general, DO and %DO saturation variances were higher at the tidal creek sites relative to the mainstem sites (Appendix 3.2).

pH:

pH is a measure of the hydrogen ion concentration of water, and is used to indicate degree of acidity. The pH scale ranges from 0 to 14 standard units (SU). A pH of 7 is considered neutral, with values less than 7 being acidic, and values greater than 7 being basic. Low pH values are found in natural waters rich in dissolved organic matter, especially in Coastal Plain swamps and black water rivers. The tannic acid released from the decomposition of vegetation causes the tea coloration of the water and low pH. Also, pH decreases with decreasing salinity.

Biota living in habitats that experience wide, rapid shifts in pH may be more susceptible to stress from contaminants because changes in pH can affect adsorption-desorption of metals and organic contaminants in sediments. Changes in pH can also affect cell membrane permeability and the function of certain enzyme systems.

For Broad Creek and the Okatee River the State Standard for pH is not lower than 6.5 or greater than 8.5.

Broad Creek:

SCDHEC Instantaneous Surface Measurements:

Tidal creek site T-4 had a measured pH of 6.45 SU (Appendix 3.1, Table 3.1). There were no other excursions beyond State Standards.

Continuous Bottom Measurements:

The only site which had pH values outside of State Standards was T-1, where values as low as 2.5 were measured (Appendices 3.2 and 3.3, Table 3.3). This pH is extremely low compared to what is normally seen, and was anomalous when compared to the rest of the earlier measurements collected at this site (Appendix 3.3). However, since the Hydrolab met all QA/QC criteria these recorded values are presented and considered valid. It is possible that the probe temporarily malfunctioned at this site. These low values recorded caused the daily average pH to be much lower than any other site and the variance to be much higher. All other values at all sites were within Standards.

Okatee River:

SCDHEC Instantaneous Surface Measurements:

The mainstem site R-6 had a measured pH of 6.2 SU and tidal creek site T-6 was 6.4 SU (Appendix 3.1, Table 3.1). There were no other excursions beyond State Standards.

Continuous Bottom Measurements:

There were no excursions beyond State Standards at any of the sites (Appendices 3.2 and 3.3, Table 3.3).

Total Organic Carbon:

Total Organic Carbon (TOC) is an indicator of the productivity of a watershed. It is a reflection of the products of decomposition of organic materials and the amount of detritus in the water column. TOC is typically greater in swamps and blackwater systems than in other waters. There are no State Standards for TOC, but comparisons can be made to values typically seen at other SCDHEC saltwater monitoring sites (SCDHEC, 1998b).

A two-way Analysis of Variance (ANOVA) indicated that, system-wide, TOC concentrations were higher in Broad Creek than in the Okatee River (Log_{10} transformed, P < 0.001). There was a significant interaction between river system and zone. Duncan's Multiple Comparison Test indicated that the elevated TOC was consistent throughout the entire Broad Creek system (no zone differences). In the Okatee River, TOC in the lower zone was lower than both the upper or middle zones. There were no habitat differences in either system, e.g. TOC concentrations were similar in tidal creeks and the mainstem of both systems (P = 0.523, two-way ANOVA).

The 1.3 inch rainfall overnight prior to the sampling of Broad Creek coupled with the extreme high tides that inundated more land area closer to upland sources than normal appears to have influenced the TOC results for Broad Creek. TOC in Broad Creek was an order of magnitude greater than any site in the Okatee River except one (Okatee River T-3, Appendix 3.1, Table 3.1). In fact, all mainstem TOC values from Broad Creek exceeded both the mean and the maximum value of 6.62 mg/l and 34 mg/l respectively measured from 1994-1998 at the SCDHEC ambient surface water quality monitoring site MD-174 in Broad Creek. The routine monitoring site is located in study segment four between study sites I-4 and R-4. All TOC concentrations in Broad Creek were greater than what is observed in 95% of all saltwater samples collected by SCDHEC from 1993-1997 (Table 3.2). TOC in the Okatee River was comparable to that seen in other SCDHEC saltwater monitoring data.

Chlorophyll-a:

Chlorophyll-a is an important pigment used by plants to capture the energy in sunlight and convert it to organic plant material through the process of photosynthesis. During the day, plants release oxygen through this process and at night, in the absence of sunlight, plant respiration consumes oxygen. These activities are responsible, in part, for the daily fluctuations in dissolved oxygen concentration. Chlorophyll-a is an indirect measurement of the amount of algal biomass at a given site. There are no State Standards for chlorophyll-a, but values between 20 ug/l and 60 mg/l were considered high in the

NOAA Estuarine Eutrophication Survey (NOAA, 1996). These values are used as a benchmark to indicate waters where there are significant algal populations that could produce nuisance algal blooms that could affect the oxygen dynamics of a system. Values greater than 60 mg/l are considered hypereutrophic (NOAA, 1996) and in imminent danger of algal related problems. These numbers were developed for open water situations and may be overprotective of smaller systems such as Broad Creek and the Okatee River.

There was a consistent effect of zone on cholorphyll-a (P = 0.022, two-way ANOVA), with higher concentrations being observed in the upper zones than the lower zones, and the middle zones having more transitional values. The upper zones of these systems are less flushed than the middle and lower zones and the longer residence times provide time for the algal populations to respond to the nutrients present.

No site in either system exceeded 60 mg/l (Appendix 3.1, Table 3.1). In Broad Creek, tidal creek sites T-2, T-3, and T-6 had chlorophyll-a concentrations exceeding 20 ug/l, as did river sites R-1 and R-2 and intertidal river site I-1 (Appendix 3.1, Table 3.1). In the Okatee River system, the chlorophyll-a concentrations at river sites R-1, R-2. and R-3 exceeded 20 ug/l, as did intertidal river site I-4 and tidal creek sites T-3 and T-4.

Nutrients:

Nitrogen and phosphorus are important plant nutrients. Under proper conditions excessive nutrients can contribute to nuisance algal blooms as well as increases in rooted aquatic vegetation. Some groups of aquatic plants and algae can "fix", or capture, nitrogen directly from the atmosphere and convert it to plant biomass. Phosphorus occurs naturally and can be released through the weathering of phosphate deposits, common in some areas of the South Carolina coast. Other sources of nitrogen and phosphorus include wastewater discharges and fertilizers, both man-made and animal wastes (manure). Phosphorus used to be common in many household detergents, but a ban on phosphate containing detergents was enacted in South Carolina in 1992. Total phosphorus (TP) and three forms of nitrogen, ammonia nitrogen (NH₃+NH₄), total Kjeldahl nitrogen (TKN), and nitrate nitrogen (NO₂+NO₃) were measured during this study. There are no State Standards for total phosphorus, total Kjeldahl nitrogen, or nitrate nitrogen (NO₂+NO₃) in saltwater, but comparisons can be made to values typically seen in other SCDHEC saltwater monitoring data (Table 3.2).

Total Phosphorus:

A two-way ANOVA indicated that total phosphorus (TP) concentrations were higher in Broad Creek than in the Okatee River (P = 0.048), and were higher in tidal creeks in both systems than at the mainstem sites. Tidal creeks seem to be functioning as conduits delivering nutrients to the main creeks.

In Broad Creek, total phosphorus concentration was high at two intertidal river sites (I-1 and I-4) and one subtidal river site (R-1, Appendix 3.1, Table 3.1). At the tidal

creek sites, five of the six sites (T-1 through T-4 and T-6) had high concentrations. In fact, four of the tidal creek sites (T-2 through T-4 and T-6) had very high concentrations.

In the Okatee River, only two tidal creeks (T-1 and T-3) had high total phosphorus concentrations (Appendix 3.1, Table 3.1). There were no values that exceeded the 95th percentile of values seen inother SCDHEC monitoring data.

In general, five of the six Broad Creek tidal creeks showed very high concentrations (0.32 - 0.72 mg/l, next highest at R-1 was 0.21 mg/l) compared to other SCDHEC saltwater monitoring data (Table 3.2). Even though the Okatee River had some high values, the highest was only 0.28 mg/l.

Ammonia:

Ammonia is a form of nitrogen, and in addition to being a potential nutrient, ammonia can be toxic to aquatic life. The State Standard for ammonia varies according to salinity, temperature and pH. Due to lab error, much of the data was lost, thirteen of thirty samples were reported as lab error or interference.

In Broad Creek, none of the samples with valid results exceeded State Standards for ammonia. One subtidal river site (R-2) was greater than 95% of the values seen in other SCDHEC monitoring data (Tables 3.1 and 3.2, Appendix 3.1). All five of the valid tidal creek samples (T-1 through T-4 and T-6) had high concentrations. In fact, four of the tidal creek sites (T-1, T-2, T-4 and T-6) had very high concentrations.

In the Okatee River, none of the samples with valid results exceeded State Standards. Five subtidal river sites (R-2 through R-6) had very high concentrations (Tables 3.1 and 3.2, Appendix 3.1). One tidal creek (T-1) had a high concentration of ammonia and one tidal creek site (T-3) was greater than 95% of the values seen in other SCDHEC monitoring data (Table 3.2).

Total Kjeldahl Nitrogen and Nitrate/Nitrite Nitrogen:

Total Kjeldahl nitrogen (TKN), and nitrate nitrogen (NO₂+NO₃) are also forms of nitrogen, and therefore potential nutrients.

In Broad Creek, TKN was high at tidal creek T-2. At tidal creek site T-4, TKN was very high and nitrate was also high. Nitrate was very high at tidal creek site T-1. All other values were comparable to values observed in other SCDHEC saltwater monitoring data (Tables 3.1 and 3.2, Appendix 3.1). In the Okatee River, TKN was very high at tidal creek site T-3. All other values were comparable to values observed in other SCDHEC saltwater monitoring data (Tables 3.1 and 3.2, Appendix 3.1).

Five-Day Biochemical Oxygen Demand (BOD₅):

Five-day Biochemical Oxygen Demand (BOD₅) is a measure of how much dissolved oxygen is consumed by the decomposition of organic matter, both natural and man-made wastes, in the water column. Although BOD₅ is strictly regulated on National Pollutant Discharge Elimination System (NPDES) permits to protect instream dissolved oxygen, there is no instream State Standard for it. However, comparisons can be made to values typically seen in other SCDHEC saltwater monitoring data (Table 3.2).

In general BOD₅ concentrations in the mainstem of each system decreased from upstream to downstream. In Broad Creek, one intertidal river and one subtidal river site (R-1 and I-1) had very high concentrations of BOD₅, as did one of the tidal creek sites (T-5, Tables 3.1 and 3.2, Appendix 3.1). In the Okatee River, one subtidal river site (R-2) had a high concentration of BOD₅ and one subtidal river site (R-1) had a very high concentration of BOD₅ (Tables 3.1 and 3.2, Appendix 3.1). Tidal Creek site T-3 also had a very high concentration of BOD₅ relative to other SCDHEC saltwater monitoring data.

Turbidity:

Turbidity is an indication of water clarity, which is actually a measurement of how light is transmitted or scattered by a water sample. Cloudy, highly turbid waters can be indicative of increased soil erosion and movement of particulate material from the land surface to a waterbody. This condition is often related to changes in land cover, e.g. clearing for new development, increased impervious surface, removal of forest or crop cover, etc. Increased turbidity can inhibit the growth of algae and rooted aquatic plants through the reduction in light intensity and may be an indicator of increased sedimentation that could impact benthic habitats. There are no saltwater State Standards for turbidity, but comparisons can be made to values typically seen in other SCDHEC saltwater monitoring data (Table 3.2).

In Broad Creek, turbidity was high at one intertidal river site (I-1) and very high at the other two intertidal river sites (I-4 and I-6, Appendix 3.1, Tables 3.1 and 3.2) relative to other SCDHEC saltwater monitoring data. Turbidity was very high at the most upstream subtidal river site (R-1). Turbidity was also very high at two tidal creek sites (T-2 and T-4). In fact, turbidity was extremely high at T-4 with a value of 200 NTU.

In the Okatee River, turbidity was high at one intertidal river site (I-4). At the subtidal river sites, turbidity was high at four subtidal river sites (R-2 and R-4 through R-6) and very high at the other two sites (R-1 and R-3). Two tidal creeks had high turbidity (T-3 and T-5) and two had very high turbidities (T-1 and T-2).

The 1.3 inch rainfall overnight prior to the sampling of Broad Creek coupled with the extreme high tides that inundated more land area closer to upland sources than normal appears to have influenced the turbidity results for Broad Creek. In fact, all mainstem turbidity values from Broad Creek exceeded the mean value of 6.87 NTU measured from

1994-1998 at the SCDHEC ambient surface water quality monitoring site MD-174 in Broad Creek. Five out of nine mainstem sites equaled or exceeded the maximum value of 25 NTU measured over the same period at MD-174. The routine monitoring site is located in study segment four between study sites I-4 and R-4.

Although the rainfall runoff to Broad Creek would have been expected to produce higher turbidities in that system than in the Okatee River system, mainstem turbidity was still greater in the Okatee River than in Broad Creek (P = 0.026, Mann Whitney Rank Sum Test).

Salinity:

Salinity and fluctuations in salinity play important roles in determining the distribution of a variety of aquatic fauna and their various life stages. Wide daily fluctuations in salinity may produce physiologically stressful conditions that exclude certain species or life stages.

In Broad Creek, the probes went dry for short periods at tidal creek sites T-1 through T-4. The mean daily bottom salinities were very similar for both the mainstem and tidal creek sites, however, variances were much greater at tidal creek sites T-1, T-3 and T-6, and somewhat greater at T-4 (Appendices 3.2 and 3.3, Table 3.3). This is due to the much lower daily minimum values. Sites T-1 and T-6 exhibited a daily salinity range greater than 20 ppt, a condition often associated with degraded benthic communities (see Chapter 5 for additional detail).

In the Okatee River, the Hydrolab record was interrupted at sites T-1, T-3 and to a lesser extent at T-6. A pattern very similar to Broad Creek was observed in the Okatee River, with tidal creek sites T-1, T-4 and T-6 showing much greater variance and lower daily minima. Sites T-1 and T-6 exhibited a daily salinity range greater than 20 ppt, a condition often associated with degraded benthic communities (see Chapter 5 for additional detail). Once again, daily mean salinity was very similar between the mainstem and tidal creek sites.

Fecal Coliform Bacteria:

Coliform bacteria are present in the digestive tract and feces of all warm-blooded animals, including humans, poultry, livestock, and wild game species. Diseases that can be transmitted to humans through water or the consumption of shellfish contaminated by improperly treated human or animal waste are the primary concern related to coliform bacteria in the environment. At present, it is difficult to distinguish between waters contaminated by animal waste and those contaminated by human waste.

Public health studies have established correlations between fecal coliform numbers in recreational, drinking, and shellfish harvesting waters and the risk of adverse human health effects. Based on these relationships, the USEPA, the Interstate Shellfish Sanitation Conference (ISSC) and SCDHEC have developed enforceable Standards for

surface waters to protect against adverse health effects from various recreational, drinking water, or shellfish consumption uses. Proper waste disposal or sewage treatment prior to discharge to surface waters minimizes this type of pollution.

Urbanization of upland areas adjacent to estuarine ecosystems has resulted in significant inputs of bacterial and chemical contaminants in salt marsh ecosystems of the southeastern US (Vernberg et al., 1993). During the pioneering stages of urban development, human waste disposal needs were met by use of septic tank based technology. As urban development proceeds and critical carrying capacity for human population density is reached, significant inputs of bacterial pollution from septic tank discharges into estuarine ecosystems may result (El-Figi, 1991), often causing closure of shellfish harvesting waters due to the presence of pathogenic bacterial/viral pollution (Leonard,1993). The normal solution to this problem is to construct a central sewer collection system to reduce estuarine inputs from individual septic tank systems (Jolley, 1978).

Vernberg et al. (1996) compared bacterial water quality in two different estuaries in South Carolina, North Inlet, a pristine NOAA National Estuarine Research Reserve and Sanctuary Site, and Murrells Inlet, a highly urbanized estuary located on the southern end of the Myrtle Beach "Grand Strand". Results indicated that 67% of the surface water monitoring stations in Murrells Inlet exceeded the Shellfish Harvesting (SFH) water quality criteria for fecal coliform bacteria (14/100ml) compared to only 33% of the stations in North Inlet. Poor water quality stations in Murrells Inlet were associated with high densities of septic tanks in close proximity to the estuary and other urban activities (marinas, boat landings and roadways). GIS overlays and statistical analysis indicated that regions in Murrells Inlet with high levels of PAHs, near roadways and marinas, also had concomitant high fecal coliform bacteria densities. This suggests that fecal coliform bacterial densities may be affected (due to biostimulation) in areas with high PAH concentrations. Poor water quality in North Inlet was associated with upland areas, where large populations of birds and wildlife reside.

Vernberg et al. (1996) also found that fecal coliform bacterial serotyping of surface waters indicated there were significant differences in the speciation of coliform positive species in surface waters of Murrells Inlet and North Inlet. In urbanized Murrells Inlet, there was a greater occurrence of *E. coli* bacteria, fewer stations that were coliform negative and a reduced number of bacterial species comprising the coliform group, particularly soil-sorbed microbes of the Pseudomonid family. In pristine North Inlet, surface waters had a greater number of coliform negative stations, reduced occurrence of *E. coli* bacteria and an increased number of bacterial species comprising the coliform group with an increased occurrence of soil-sorbed microbes in the Pseudomonad family. The greater diversity/species richness in the coliform group members in North Inlet resulted from the availability of bacteria from the deciduous hardwood forest when compared to upland watersheds in urbanized Murrells Inlet, which contain more monoculture (i.e. lawns with grass and ornamental plants) habitat. These findings clearly indicate that fecal coliform bacteria pollution is associated with urbanization and that

closure of shellfish harvesting waters may be perhaps the most significant, quantifiable impact from urbanization.

The current fecal coliform bacterial assay is unable to discern between coliform bacteria from human versus animal sources. While both animal and human sources of bacterial pollution may be a significant human health threat, differentiating between sources is critical in formulating effective environmental management strategies to reduce loading from bacterial pollution sources. Several techniques have been evaluated to potentially discriminate between animal and human sources including Pulsed Field Gel Electrophoresis (PFGE), Fatty Acid Profiling (FAP), ribotyping, Analytical Profiling Index (API) biotyping and Multiple Antibiotic Resistance (MAR) testing. As part of this study Analytical Profiling Index (API) biotyping and Multiple Antibiotic Resistance (MAR) testing were employed to try to determine the probable source(s) of the observed coliforms. In addition, the coliform isolates used in these tests will subsequently be subjected to Pulsed Field Gel Electrophoresis (PFGE) and ribotyping, the results of which will be reported in a separate report.

The positive fecal coliform Most Probable Numbers (MPN) sample results obtained by SCDHEC were transferred to the NOS Center for Coastal Environmental Health and Biomolecular Research at Fort Johnson, SC, for further speciation and biotyping. At the NOS-CCEHBR facility these samples were streaked onto selective agar and different members of the coliform group are identified with Analytical Profiling Index methodologies. *E. coli* bacteria are selectively isolated by this method.

Standards Compliance

The State Standard (SCDHEC, 1998a) for fecal coliform bacteria in Broad Creek and the Okatee River, to protect primary contact recreation (swimming), is a geometric mean that does not exceed 200 colonies/100 ml based on five consecutive samples in a 30 day period, and no more than 10% of the total number of samples collected in a 30 day period can exceed 400 colonies/100 ml. To protect for the consumption of shellfish the MPN fecal coliform geometric mean shall not exceed 14 colonies/100 ml, nor shall more than 10% of the samples exceed an MPN of 43 colonies/100 ml (SCDHEC, 1998a).

The analytical method required for the determination of recreational Standard compliance and shellfish harvesting status are different and mutually exclusive. The method employed in this study conformed to USEPA accepted methods, required through State Standards (SCDHEC, 1998a) for water quality evaluation and do not satisfy the methodological requirements for the determination of shellfish harvesting classification.

Because only a single sample was collected at each site, compliance with the Standards cannot be strictly determined, but the observed data can still be compared to the Standard for informational purposes only.

System wide, fecal coliform bacteria concentrations were higher in Broad Creek than in the Okatee River (Log₁₀ transformed, P < 0.001, T-Test, Figure 3.5). Okatee

River tidal creeks were not significantly different from mainstem Okatee River sites. In Broad Creek, fecal coliform bacteria concentrations in tidal creek sites were higher than in the mainstem sites (P = 0.007, Mann Whitney Rank Sum Test). Fecal coliform bacteria concentrations in Broad Creek tidal creeks were also higher than in Okatee River tidal creeks (P = 0.015, Mann Whitney Rank Sum Test). When the data from both systems were pooled, tidal creek bacteria concentrations were higher than mainstem concentrations (Log_{10} transformed, P = 0.006, T-Test), suggesting that tidal creeks may be acting as conduits to deliver bacteria from the uplands to the mainstem areas. The occcurrence of elevated fecal coliform bacteria concentrations in drainage ditches and the canal systems draining to Broad Creek has been previously documented (SCDHEC, 1996). In Broad Creek, seven sites (46.7%) had MPNs > than the upper detection limits of the test (e.g. > 1600 colonies/100 ml) versus only 1 site (7%) in the Okatee River (Appendix 3.1, Table 3.1).

Broad Creek:

Shellfish Standards: Concentrations greater than 14 colonies/100 ml were measured at all sites (Figures 3.6 and 3.7, Appendix 3.1, table 3.1). In the mainstem, all three intertidal river sites exceeded 43 colonies/100 ml, and four of the six river sites (R-1, and R-4 through R-6) exceeded 43 colonies/100 ml. In the tidal creeks, all values exceeded 43 colonies/100 ml.

Recreational Standards: Concentrations greater than 200 colonies/100 ml were measured at one intertidal river site (I-4), one river site (R-4) and all tidal creek sites (Figures 3.6 and 3.7, Appendix 3.1, Table 3.1). Similarly, R-4 and all tidal creek sites exceeded 400 colonies/100 ml.

Okatee River:

Shellfish Standards: On the mainstem (intertidal river and subtidal river sites), concentrations greater than 14 colonies/100 ml were measured at only two sites, R-1 and R-2, and only one site, R-1, exceeded 43 colonies/100 ml (Figures 3.6 and 3.7, Appendix 3.1, Table 3.1). Five of the tidal creek sites exceeded 14 colonies/100 ml, T-1 through T-5. Of these, only T-1 and T-3 exceeded 43 colonies/100 ml.

Recreational Standards: None of the mainstem (intertidal river and subtidal river sites) sites exceeded 200 colonies/100 ml (Figures 3.6 and 3.7, Appendix 3.1. Table 3.1). Only tidal creek sites T-1 and T-3 exceeded 200 colonies/100 ml, and T-3 was the only site that exceeded 400 colonies/100 ml.

Biotyping Evaluation

The objectives of this portion of the study was to evaluate impacts of urbanization on bacterial water quality in Broad Creek and the Okatee River and to apply new novel techniques such as MAR to potentially determine pollution sources within each watershed.

E. coli is the primary fecal coliform bacterium present in human and animal feces. It is considered the indisputable fecal contamination indicator for warm-blooded animals (Kator and Rhodes, 1994). E. coli has been proposed as a replacement indicator for fecal coliforms because of the pervasive presence of other coliform members such as Klebsiella, which are not specifically associated with human fecal contamination. Vernberg et al. (1996) reported that the second most dominant member of the fecal coliform group behind E. coli was Klebsiella pneumoniae in evaluation of estuarine surface waters and oysters from pristine and urban estuaries within SC.

Multiple Antibiotic Resistance (MAR) is a novel technique proposed as a new method for differentiating human versus animal fecal pollution sources (Parveen et al., 1997). This approach is based upon the fact that E. coli from wildlife species are lacking in antibiotic resistance while strains from humans will exhibit MAR. Strains from domestic animals will be intermediate in MAR. Parveen et al., (1997) used MAR to 10 antibiotics to evaluate 765 E. coli isolates from surface water samples collected from regions of Apalachicola Bay estuary in Florida impacted by point source bacterial pollution (sewage treatment plants) and areas impacted by NPS runoff. Results indicated that 82% of all samples were resistant to one or more antibiotics. The MAR Index was reduced by 50% in comparisons of point and NPS impacted areas. Application of MAR to watersheds in SC would be useful in differentiating human versus wildlife fecal pollution sources. Scott and co-workers at NOAA have used MAR profiling on selected watersheds in SC including the Isle of Palms and 10 different sewage treatment plants. Results have generally indicated significantly higher MAR in urban versus pristine watersheds and similar MAR Index values as reported by Tamplin in urban and pristine areas, dominated by NPS runoff from wildlife.

Methods:

Fecal coliform densities were determined by SCDHEC for the 30 field sites (Broad Creek and Okatee River) and seven sewage treatment plants (Forest Trails = FTSTP; Broad Creek = BC1; Hilton Head = HH1; Long Cove Creek = LC1; Okatee River = OK1; SI = South Island and Wexford Sound = WX1) using the 3 tube MPN method as described by APHA (1984). Samples testing positive for fecal coliforms were then further isolated using selective Violet Red Bile Agar (VRBA). Ten presumptive *E. coli* isolates from each plate, or 15 isolates from the sewage treatment plants, were then picked and inoculated on to Plate Count Agar (PCA) for isolation. Each coliform isolate was identified to genus and species by the Analytical Profiling Index methods (API 20 E test kit bioMerieux). The results for the 10 or 15 individual isolates/sample were statistically analyzed (cumulative proportions) and the proportional distributions of each species comprising the coliform group were determined. Different *E. coli* strains were further identified using the API procedure. An overview of these methods is depicted in Figure 3.8.

Confirmed *E. coli* isolates were tested for multiple antibiotic resistance (MAR) using methods described by Parveen et al. (1997). Each *E. coli* isolate was tested on agar

plates containing one of 10 antibiotics, or a control plate without antibiotics. The 10 antibiotics included: ampicillin (10 ul/ml), chlortetracycline (25 ul/ml), kanamycin (25 ul/ml), nalidixic acid (25 ul/ml), neomycin (50 ul/ml), oxytetracycline (50 ul/ml), penicillin G (75 ul/ml), streptomycin (12.5 ul/ml), sulfathiozole (500 ul/ml), and tetracycline (25 ul/ml).

Resulting growth in each antibiotic was compared to growth on a control (no antibiotic) plate. Antibiotic Resistance for each antibiotic was defined as a growth inhibition of <15% in size when compared to the control plate. Sensitivity was defined as growth inhibition that was >15% in size when compared to the control plate. Other MAR end-points measured included: 1) MAR Index (%) for an Isolate = (# antibiotics to which an isolate was resistant/total # antibiotics tested); 2) Site MAR Index = (# antibiotics to which all isolates were resistant/total # antibiotics tested multiplied by the number of isolates/site); and 3) Total # of Antibiotics Resistant = (Total # Antibiotics Resistant for all isolates/site). An overview of these methods is depicted in Figure 3.8.

Results:

Results of API biotyping (Figures 3.9-3.11) indicated that positive fecal coliform samples were comprised primarily of *E. coli* in both Broad Creek and the Okatee River. In Broad Creek (Figure 3.9), all stations (100%) contained *E. coli* compared to only 73% of the sites in the Okatee River (Figure 3.10). In Broad Creek, in addition to *E. coli*, the other species observed included *Klebsiella pneumoniae* (13.3% of the sites), *Klebsiella oxytoca* (6.7%), *Enterobacter sakazaki* (6.7%), *Enterobacter aerogenes* (6.7%) and unknown bacterial species (33% of the sites). In the Okatee River, in addition to *E. coli*, the other species observed included *Klebsiella pneumoniae* (13.3%), *Enterobacter vulnaris* (13.3%), and unknown bacterial species (6.7%). In addition, 13.3% of the sites were coliform free (MPN < 2/100 ml).

The mean overall API coliform "finger prints" for Broad Creek and the Okatee River are depicted in Figure 3.11, along with comparisons from pristine North Inlet (a NOAA National Estuarine Research Reserve and Sanctuary Site) and a highly urbanized, Murrells Inlet Site, located just south of the Myrtle Beach Grand Strand. Note that the mean distribution of E. coli in the Okatee River (66%) was just slightly higher than that measured in pristine North Inlet (53%). Similarly, there was a much higher occurrence of E. coli in urbanized areas such as Broad Creek (90%) and Murrells Inlet (83%) and the percentage of coliform free sites decreased with increased urbanization (North Inlet --> Okatee River ---> Murrells Inlet ---> Broad Creek). In highly urban areas, the slightly lower occurrence of E. coli and small coliform free areas in Murrells Inlet (when compared to Broad Creek) resulted from a green space (undeveloped region) corridor located at the southern (Huntington Beach State Park) end of the estuary. In Broad Creek, a lack of planned green space corridors has resulted in 100% occurrence of E. coli bacteria throughout the entire tidal creek watershed. Open spaces in the Okatee River have resulted in a large potion of that watershed where E. coli and other coliform bacteria do not occur. The inclusion of green space corridors is important in maintaining the assimilative capacity of tidal creek watersheds.

Analysis of the different *E. coli* bacteria biotypes found at each site (Figure 3.12-3.13) indicated that there were slight differences in the proportion of different *E. coli* types between Broad Creek and the Okatee River. In the Okatee River (Figure 3.13), *E. coli* strain 5144572 was the dominant strain accounting for 69.7% of all *E. coli* present. Note the dominance of this *E. coli* strain at tidal creek, river and intertidal river sites in the Okatee River. The second most dominant strain was *E. coli* type 5044572 (15.1%) followed by *E. coli* type 5144552 (11.1%).

In Broad Creek (Figure 3.12), *E. coli* strain 5144572 was the dominant strain accounting for 35.9% of all *E. coli* present, followed closely by *E. coli* strains 5144552 (32.1%). The third most dominant strain was *E. coli* strain 5044572 (15.3%). Note the more diverse and equitable nature of these three *E. coli* strains at tidal creek, river and intertidal river sites of Broad Creek.

Comparisons of *E. coli* strains in Broad Creek and the Okatee River indicated a clear dominance of one strain (5144572) in the less developed Okatee River watershed compared to co-dominance by three strains (5144572, 5144552 and 5044572) in more urbanized Broad Creek (Figure 3.14). Richards (Dr. Gary Richards, U. S. Department of Agriculture, Delaware State University) has similarly found differential *E. coli* strains in analysis of urban versus rural areas of South Carolina.

Results of Multiple Antibiotic Resistance (MAR) testing indicated that *E. coli* bacteria in Broad Creek had greater resistance to a larger number of antibiotics than the Okatee River (Figures 3.15-3.19 and Tables 3.4-3.6). A total of 7 sites (47%) in Broad Creek tested positive for antibiotic resistance, including 50% of the tidal creek, 50% of the river and 33% of the intertidal river stations (Figure 3.15). The remainder of the Broad Creek sites (53%) tested negative for MAR. In the Okatee River, only three sites (20%) tested positive for antibiotic resistance, including 33% of the tidal creek, none of the river and 33% of the intertidal river stations (Figure 3.15, Table 3.4). The remainder of the Okatee River sites (80%) tested negative for MAR. Site MARs ranged from 0-26.4%, averaging 3.4% in Broad Creek versus a range of 0-5.71%, averaging 1.04% in the Okatee River (Table 3.4). This 69% reduction in MARs was very similar to MAR reductions seen in comparison of developed and undeveloped watersheds in Florida of 47% (Parveen et al., 1997) and in Maryland of 69% (Kaspar et al., 1990) (Table 3.5).

Similarly, the percentage of *E. coli* antibiotic sensitivity was higher in the Okatee River when compared to Broad Creek (Figure 3.16). Most of the tidal creek (67%), river (100%) and intertidal river (67%) stations on the Okatee River had antibiotic sensitive *E. coli* strains, ranging from 73.6-100% sensitivity. Some 1050 out of the 1061 *E. coli* isolates found in the Okatee River (99%) were sensitive to the 10 antibiotics tested (Table 3.4). In Broad Creek, most of the tidal creek (67%), river (50%) and intertidal river (50%) stations had antibiotic sensitive *E. coli* strains, ranging from 50-100% sensitivity. A total of 1394 out of the 1443 *E. coli* isolates in Broad Creek (97%) were sensitive to the 10 antibiotics tested (Table 3.4).

The number of antibiotics to which each MAR positive *E. coli* strain was resistant was greater at several sites in Broad Creek than in the Okatee River (Figure 3.17). In Broad Creek, at sites with positive MARs, the number of antibiotics each strain was resistant to ranged from 1 to 4 antibiotics, averaging 2.14 antibiotics/strain. The dominant antibiotics for MAR in Broad Creek were chlortetracycline, oxytetracycline, and tetracycline (Table 3.4). In the Okatee River at sites with positive MARs, the number of antibiotics each strain was resistant to ranged from 1 to 2 antibiotics, averaging 1.3 antibiotics/strain (Figure 3.17). The dominant antibiotic for MAR in the Okatee River was penicillin G (Table 3.4).

At sewage treatment plants (STPs) sampled in Beaufort County (Tables 3.4 and 3.6), 860 *E. coli* isolates were tested from seven different STPs. A total of 106 isolates (12.3%) were antibiotic resistant, with the dominant MAR resistance found for ampicillin (2%), penicillin G (5%) and the tetracyclines (0.7-1%). The overall MAR Index for individual STPs ranged from 5-22% (Table 3.6), averaging 12.33% (Table 3.4). Note that the highest STP site MAR of 22% for BC-1 (Table 3.6), was quite similar to the highest MAR measured in surface waters at Broad Creek of 26.4% at site R-4 (Figure 3.15). At STPs, the number of sensitive isolates was 754 out of the 860 isolates (88%) (Table 3.4). The number of antibiotics each positive MAR *E. coli* strain was resistant to at STPs ranged from 1-8 antibiotics, averaging 4.7 antibiotics/strain (Table 3.6). At STPs serving the retirement community of Hilton Head Island (BC-1, HH-1 and LC-1) the total number of antibiotics each *E. coli* strain was resistant to ranged from 7-8 antibiotics/strain versus a range of 1-3 antibiotics/strain in other STPs serving the more general population of Beaufort County (OK-1, SI-1 and Wx-1) (Table 3.6).

Comparisons of MAR and MPN results and GIS analysis of land use in each watershed indicated a clear association of areas with high MPNs, high MARs and obvious pollution sources (STPs and septic tanks) in Broad Creek (Figure 3.18). Three distinct regions of high MPNs and MAR were found in Broad Creek including stations I-1 and T-2 (septic tanks), stations T-4, R-4 and R-5 (septic tanks) and stations R-6 and T-6 (STPs land based discharges). In the Okatee River, two regions of high MAR were found at stations T-3 and I-4 (STP land based discharges) and station T-2 (unknown source) (Figure 3.19). The majority of stations in both Broad Creek (53.3%) and the Okatee River (80%) were negative for MAR, suggesting animal pollution sources rather than human pollution sources *per se*. This indicates water quality management strategies must be focused to reduce bacterial loadings from both human and animal sources. Both human and animal pollution sources must be managed, including wildlife, domestic animals and pets. The specific regions with high MARs in each watershed appear to have high spatial correlation with land based human pollution sources.

Water Quality Summary

In an effort to summarize the water quality data, a composite summary score for overall water quality was calculated at each site. This summary score was a total of the number of individual parameters exceeding predetermined threshold values (Table 3.7). Each parameter received a score of one for each of twelve total categories based on the following thresholds: 24-hour average DO less than 5.0 mg/l, fecal coliform bacteria greater than the shellfish Standard of 43 colonies per 100 ml, pH less than 6.5 SU or greater than 8.5, chlorophyll-a greater than 20 mg/l, 24-hour range in salinity greater than 20 ppt, and values exceeding the statewide saltwater 90th percentile for turbitidy, BOD, alkalinity, TKN, nitrate, TP, and TOC. If six or more of the parameters exceeded the established thresholds the site was classified as poor. If three to five parameters exceeded the thresholds a site was classified as fair, and if less than three parameters exceeded threshold levels a site was classified as good.

In Broad Creek, seven of fifteen sites scored as poor, including five of the six tidal creek sites, and six sites scored as fair (Figure 3.20). Only two sites scored as good. TOC and fecal coliform bacteria were elevated system-wide in Broad Creek. Tidal creeks were characterized by low DO, and elevated fecal coliform bacteria and total phosphorus concentrations. The 1.3 inch rainfall overnight prior to the sampling of Broad Creek coupled with the extreme high tides that inundated more land area closer to upland sources than normal appears to have influenced the sampling results for some parameters in Broad Creek, especially TOC.

In contrast, in the Okatee River, only one of fifteen sites scored as poor and nine scored as good (Figure 3.20). Only five sites scored as fair. Turbidity was significantly higher in the Okatee River than in Broad Creek. Tidal creek T-3 in the Okatee River system stands out as the only site receiving a water quality rating of poor. Fecal coliform bacteria at this site included colonies identified as having human sources based on high MAR results.

In general, DO did not meet State Standards. Standards are goals used to set permit limits, not necessarily reflective of natural conditions. Daily mean DO less than 5.0 mg/l was measured routinely in both systems. Only R-6 in Broad Creek, and R-1, R-2, R-5, R-6 and T-5 in the Okatee River met the 24-hour average of 5.0 mg/l. Two tidal creek sites in Broad Creek were classed as degraded based on DO using EMAP protocols, creeks T-4 & T-5.

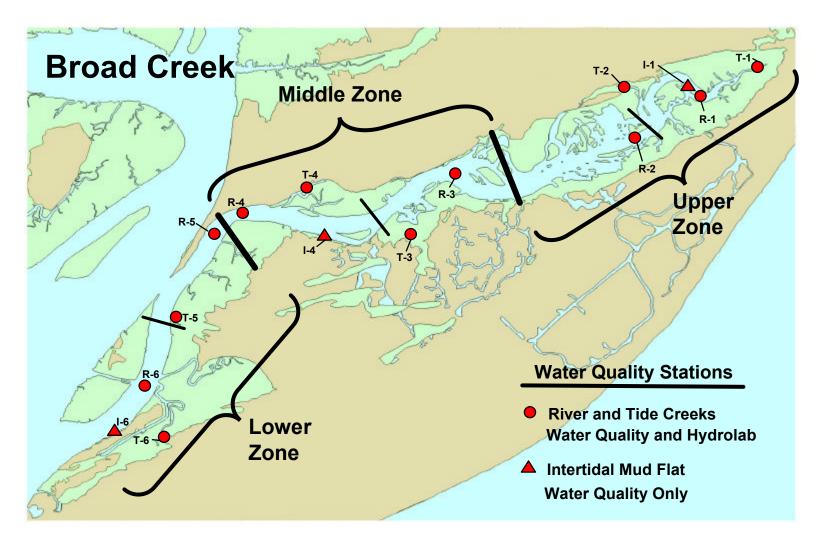


Figure 3.1. Map of Broad Creek stations sampled for water quality and fecal coliform bacteria.

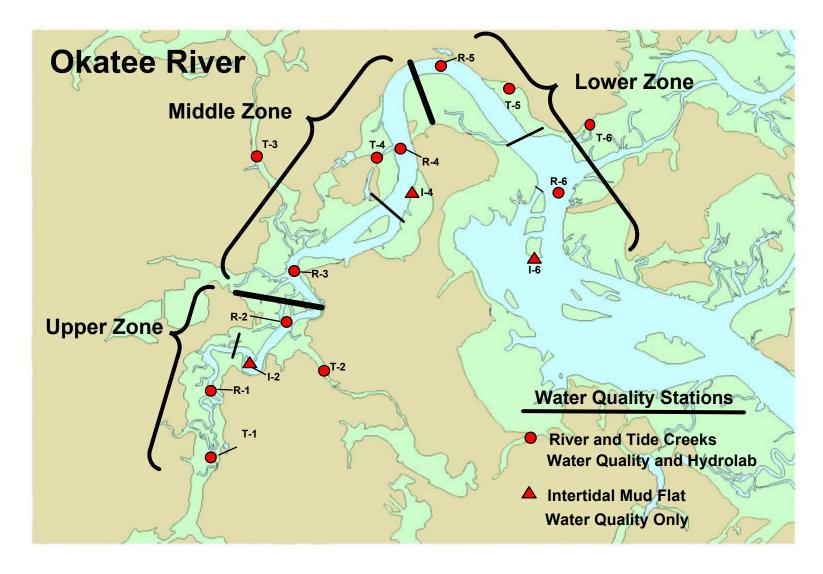


Figure 3.2. Map of Okatee River stations sampled for water quality and fecal coliform bacteria.

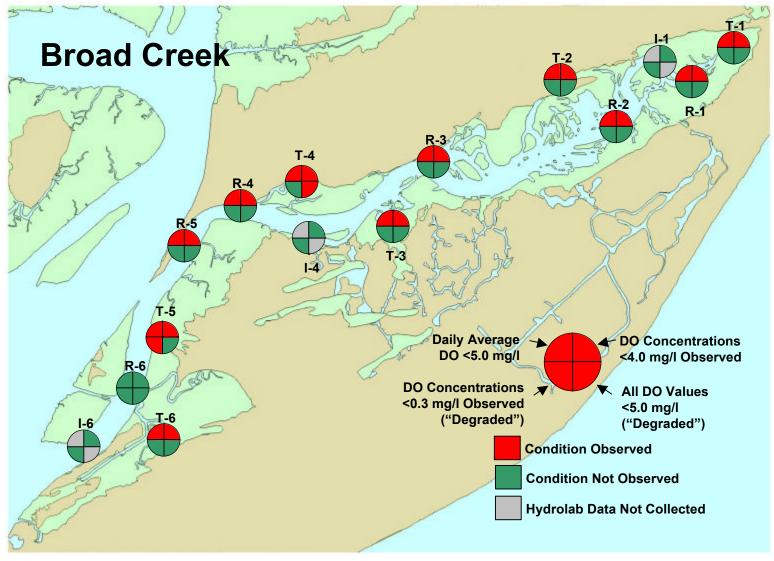


Figure 3.3. Summary of dissolved oxygen data. Where Hydrolab data were not collected, the instantaneous measurements <4.0 mg/l or <0.3 mg/l are graphed.

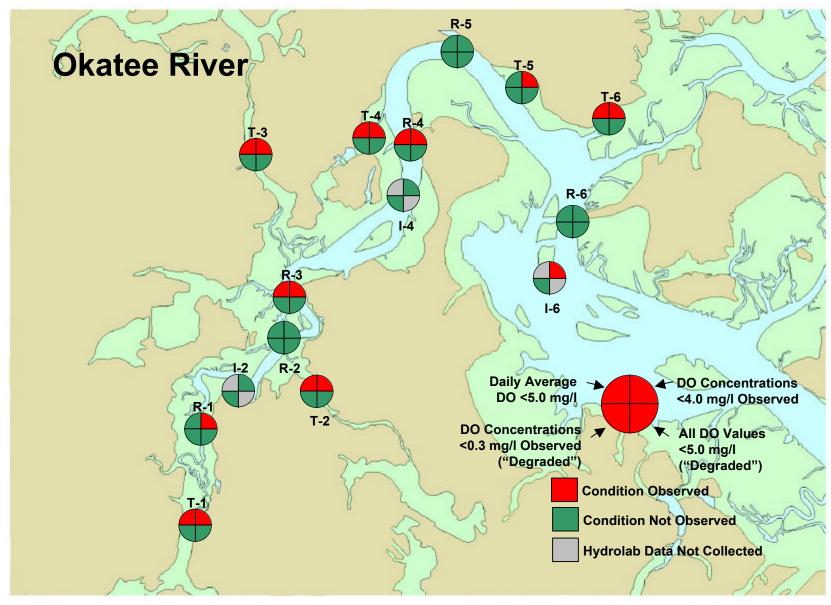


Figure 3.4. Summary of dissolved oxygen data. Where Hydrolab data were not collected, the instantaneous measurements <4.0 mg/l or <0.3 mg/l are graphed.

Coliform Bacteria

Most Probable Number (MPN)

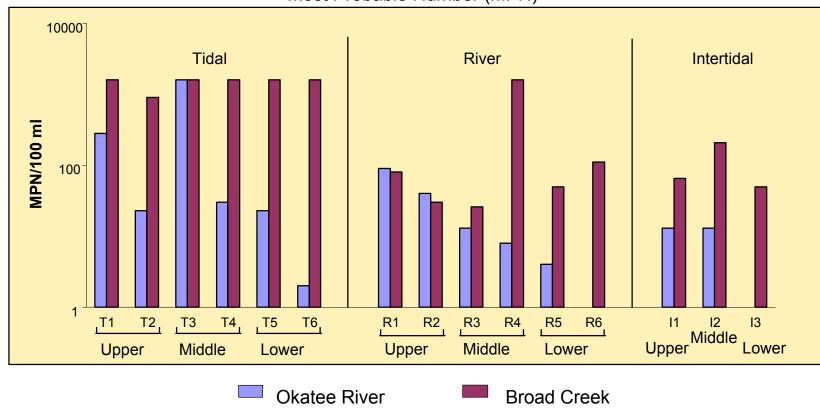


Figure 3.5. Results of fecal coliform Most Probable Numbers (MPNs) measured in Broad Creek and the Okatee River. Note the much higher MPNs in Broad Creek than in the Okatee River at most habitats tested (tidal creeks, river and intertidal sites).

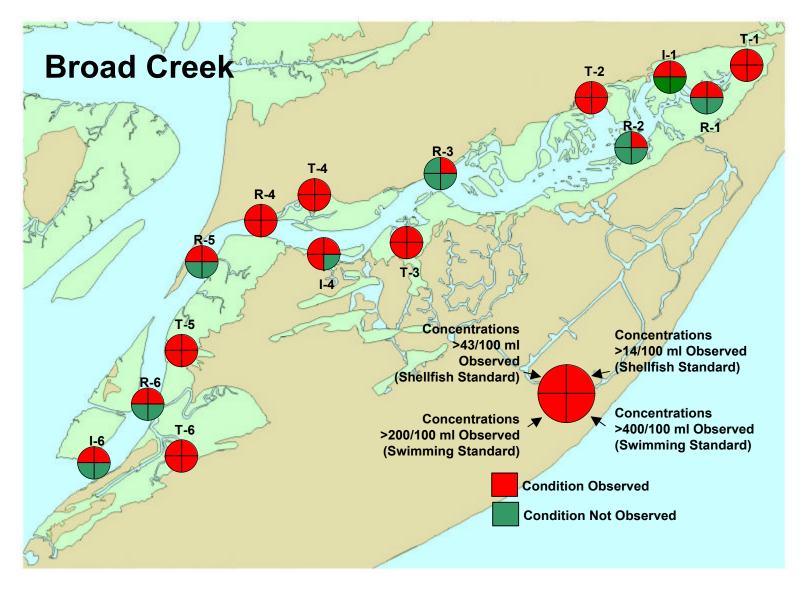


Figure 3.6. Summary of fecal coliform bacteria data for Broad Creek.

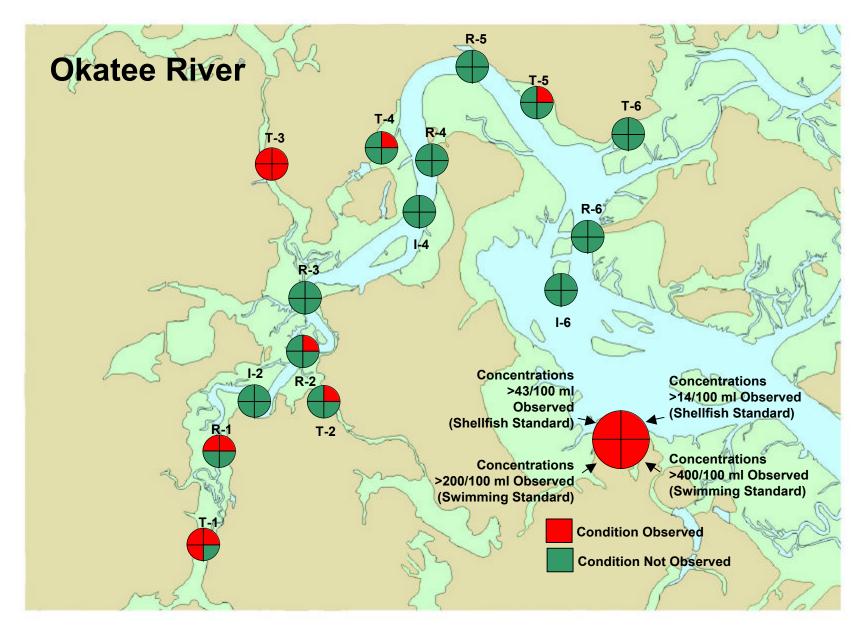
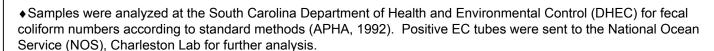


Figure 3.7. Summary of fecal coliform bacteria data for the Okatee River.

METHODS

Sample collection and preparation

♦ One sample from each site was collected in a sterile plastic jar in August, 1997.





♦A total of 10 isolates from violet red bile agar plates were selected from each surface water sample and 15 isolates were chosen from each of the STP samples.



◆The identification of each isolate was confirmed as E. coli by API 20 E test kit (bioMerieux Vitek, Hazelwood, MO).



Multiple Antibiotic Resistance:

♦ Confirmed *E. coli* isolates were further tested for MAR following the method of Parveen et al., (1997), briefly described below.



♦ Isolates were transferred to a 96 well plate containing tryptic soy broth (TSB) and incubated for 4-6 h at 35 °C.



◆The broth cultures were then transferred in duplicate with a 48-prong replicator to Mueller-Hinton agar plates, each containing one of 10 antibiotics, or to a control plate without antibiotics. Plates were incubated 18-24 hours at 35 ° C.



- ♦ The antibiotics and their concentrations in agar were: ampicillin, 10 μl/ml; chlortetracycline, 25 μl/ml; kanamycin μl/ml, 25 μl/ml; nalidixic acid, 25 μl/ml; neomycin, 50 μl/ml; oxytetracycline, 50 μl/ml; penicillin G, 75U/ml; streptomycin, 12.5 μl/ml; sulfathiazole, 500 μl/ml and tetracycline, 25 μl/ml.
- ◆Resulting growth was measured and compared to the size of the same isolate on the control plate. **Resistance** = less than 15% reduction in colony size on the antibiotic plate compared to the control plate. **Sensitivity** = greater than or equal to 15% reduction in size compared to the control plate. Also calculated were:



- ♦ MAR index (%) for an isolate = (# of antibiotics to which the isolate was resistant / total # of antibiotics tested) × 100.
- ◆ MAR index (%) for a sample site = # of antibiotics to which all the isolates were resistant / (number of antibiotics tested × number of isolates per site) × 100.

Figure 3.8. Overview of MPN, API Biotyping and MAR methodologies used in this study.

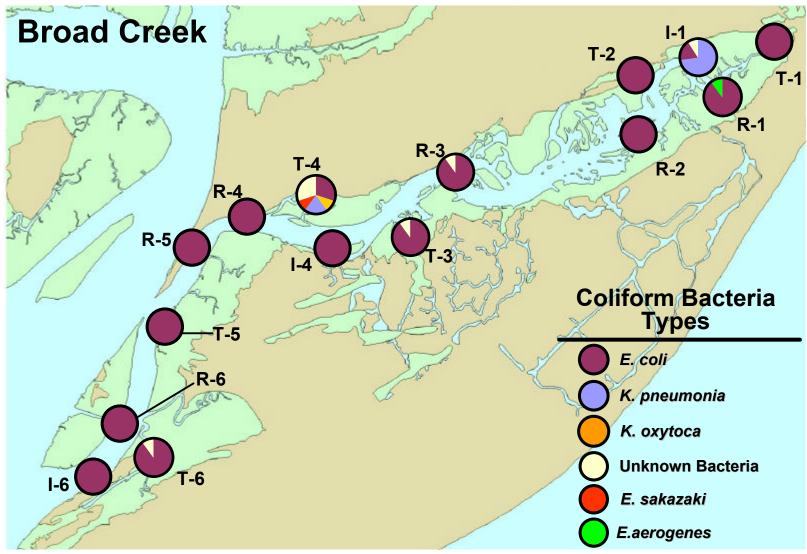


Figure 3.9. Results of API Biotyping measured at each site in Broad Creek. Note that *E. coli* was the dominant fecal coliform bacteria measured at all sites (100%) and that no sites were free of coliform bacteria.

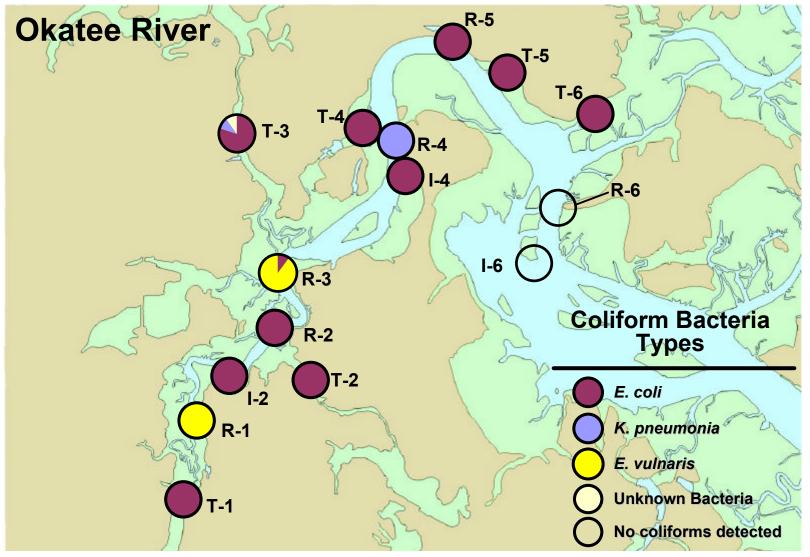


Figure 3.10. Results of API Biotyping measured at each site in the Okatee River. Note that *E. coli* was measured at only 73.3% sites and was the dominant fecal coliform bacteria, and that 13.3% of the sites were free of coliform bacteria.

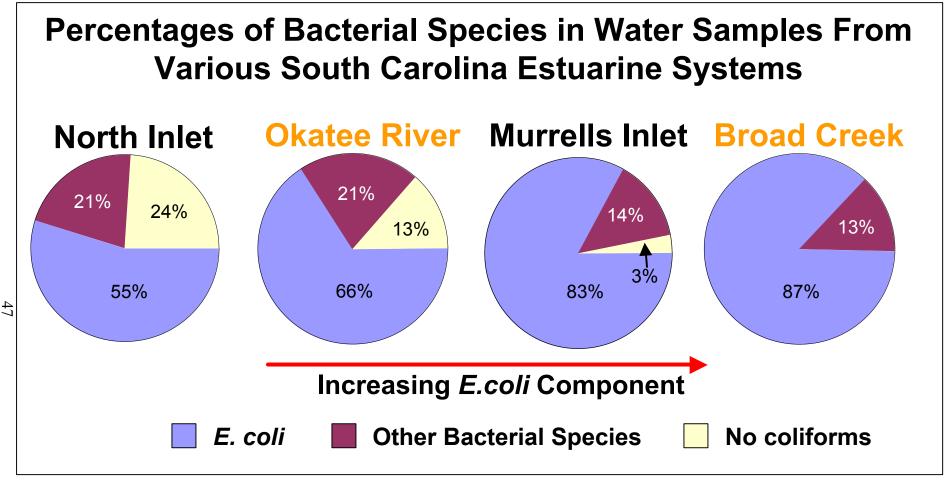


Figure 3.11. Comparison of mean API Biotyping results for all sites in Broad Creek and the Okatee River. Also included for comparison is North Inlet, a pristine NOAA National Estuarine Research Reserve and Sanctuary Site and urbanized Murrells Inlet, located on the southern end of the Myrtle Beach "Grand Strand". Note the increased occurrence of *E. coli* bacteria, as the dominant member of the coliform group and decreased proportion of sites with coliform free samples with increased urbanization. In Broad Creek all sites contained *E. coli* bacteria (100%) versus 73.3% in the Okatee River, a rural area with single family dwellings.

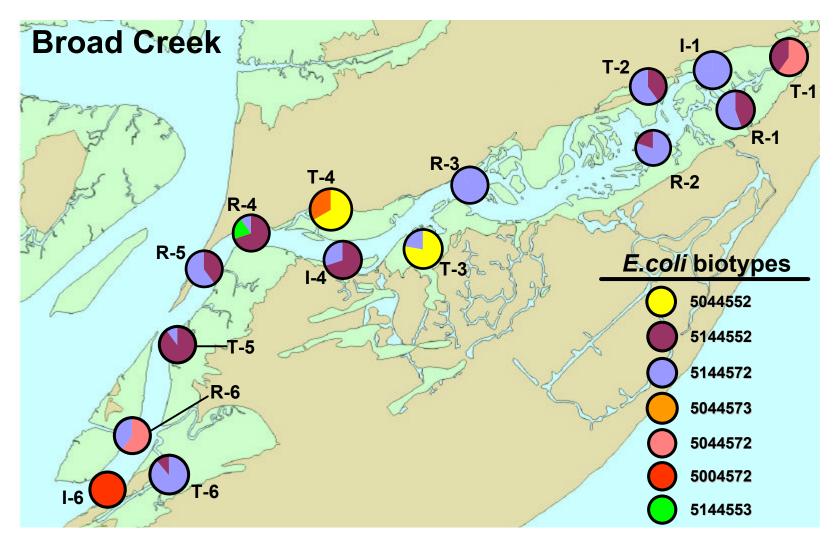


Figure 3.12. *E. coli* bacteria biotypes measured in Broad Creek. Note that API Code 5144572, while the dominant *E. coli* bacteria found in Broad Creek at a majority of the stations sampled (35.9%), was much less prevalent than in the Okatee River (69.7%), and there was much more equitability with several other *E. coli* strains, including 5144552 (32.1%) and 5044572 (15.3%).

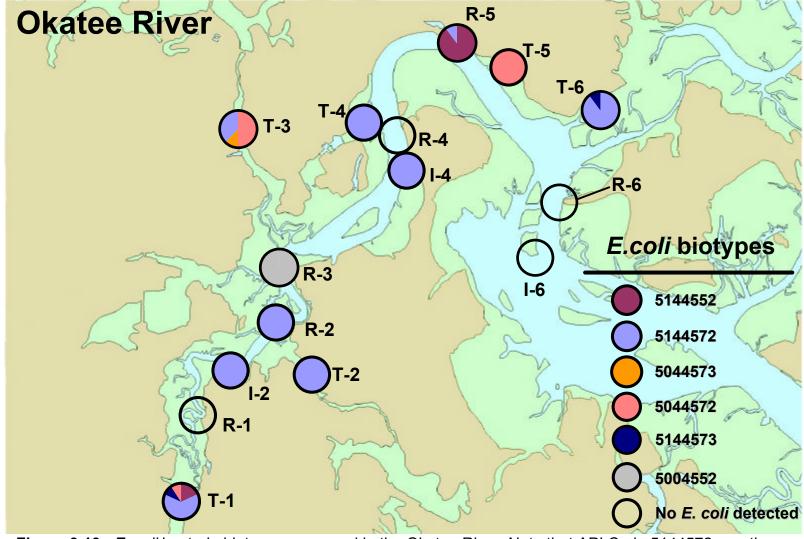


Figure 3.13. *E. coli* bacteria biotypes measured in the Okatee River. Note that API Code 5144572 was the dominant *E. coli* bacteria found in Okatee River at a majority of the stations sampled (69.7%). There was clear dominance by this one API code of *E. coli* in the Okatee River.

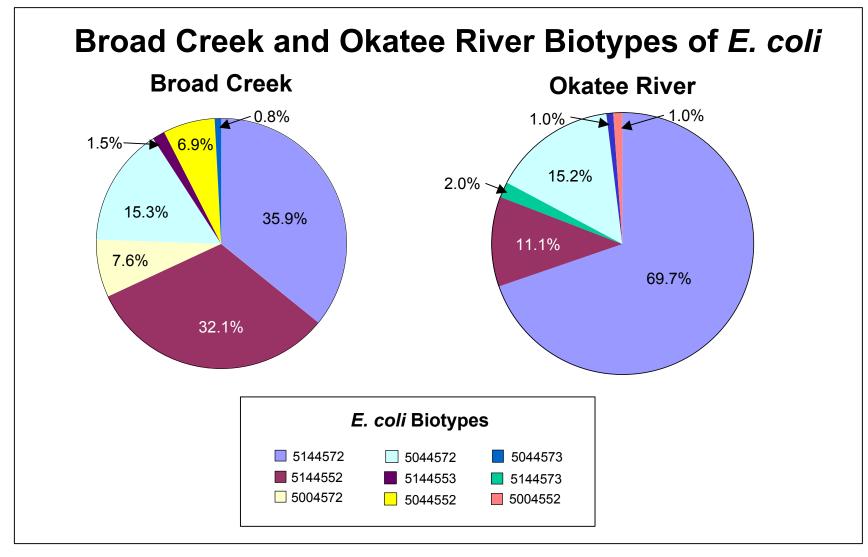


Figure 3.14. A comparison of mean *E. coli* API Codes measured in Broad Creek and the Okatee River. Note the much higher prevalence rate of API code 5144572 in the Okatee River (69.7%) when compared with Broad Creek (35.9%), and the equitability of API codes 5144552 (32.1%) and 5044572 (15.3%) in Broad Creek.

Multiple Antibiotic Resistance (MAR)

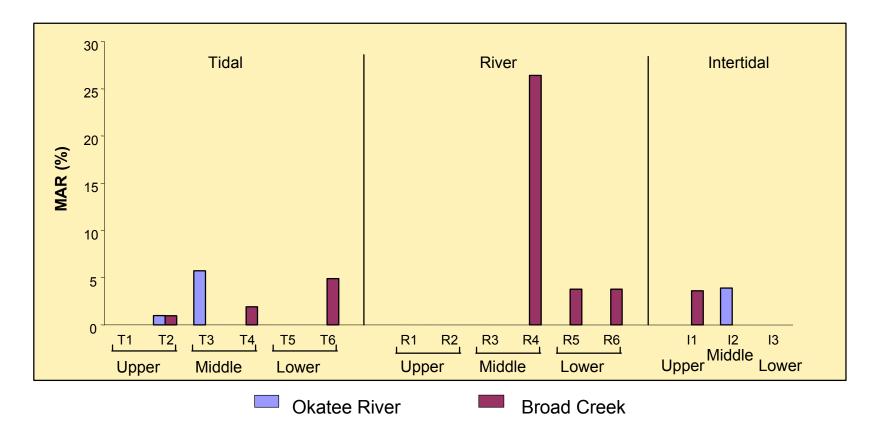


Figure 3.15. Multiple Antibiotic Resistance (MAR) Index for each site in Broad Creek and the Okatee River. Note the much higher MAR Index at numerous sites in Broad Creek (n=7 sites) when compared to the Okatee River (n=3 sites).

Antibiotic Sensitivity

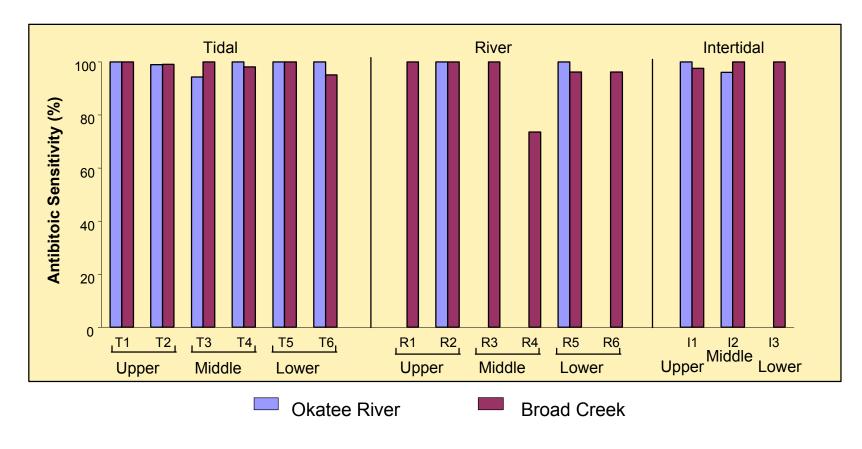


Figure 3.16. MAR Sensitivity for each site in Broad Creek and the Okatee River.

Antibiotic Resistance

Number of Resistant Antibiotics per Site

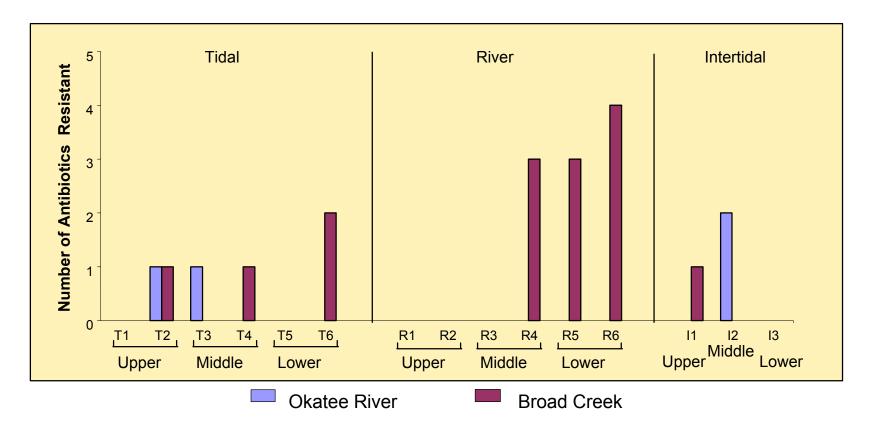


Figure 3.17. A comparison of the number of antibiotics each MAR *E. coli* strain was resistant to in Broad Creek and the Okatee River. Note that the number of resistant antibiotics/strain was much higher in Broad Creek (1-4 resistant antibiotics/strain,averaging 2.14 resistant antibiotics/strain) than in the Okatee River (only 1-2 resistant antibiotics/strain).

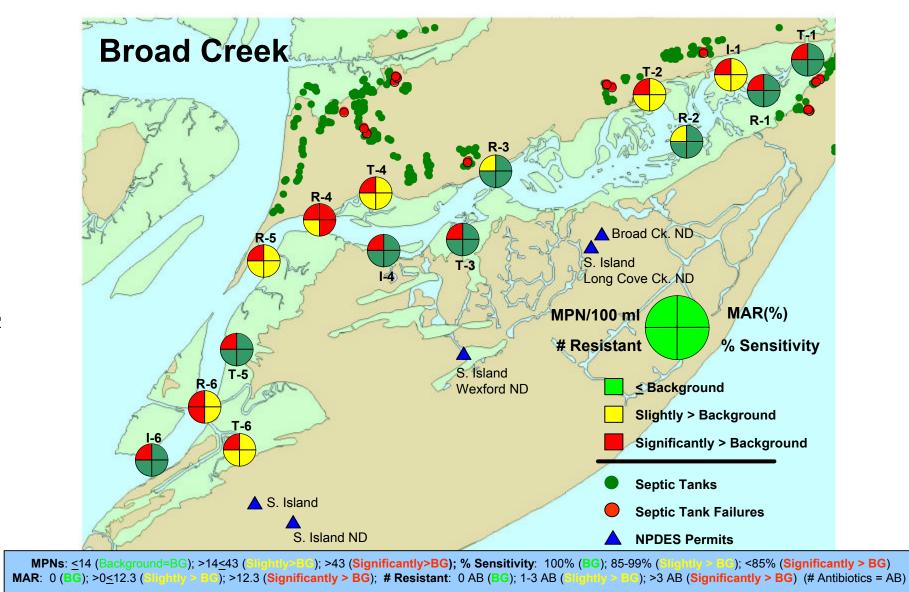


Figure 3.18. Coliform bacteria and multiple antibiotic resistance in Broad Creek. Septic tanks (green and red dots) and permitted wastewater disposal sites (blue triangles) indicate a possible correlation of MAR results with wastewater management practices within this region.

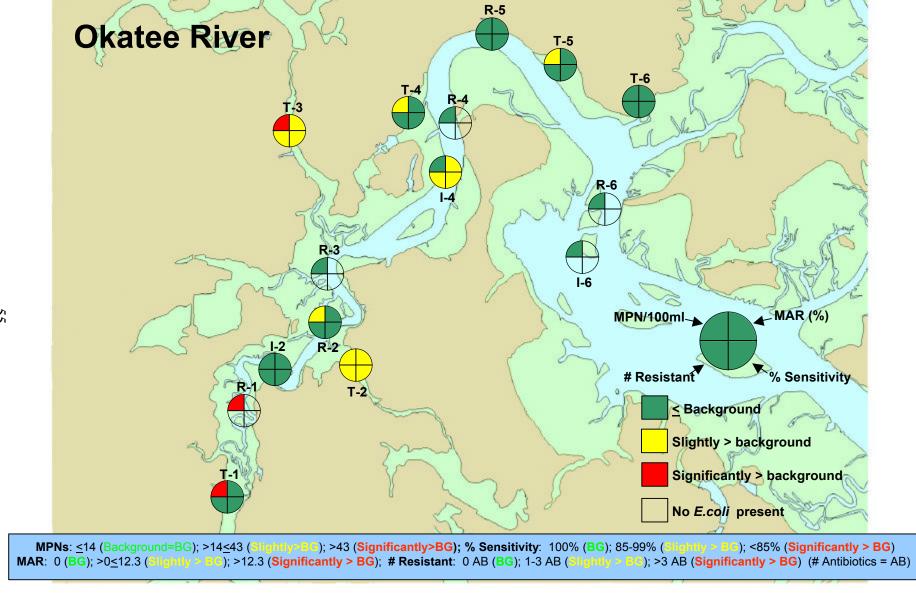
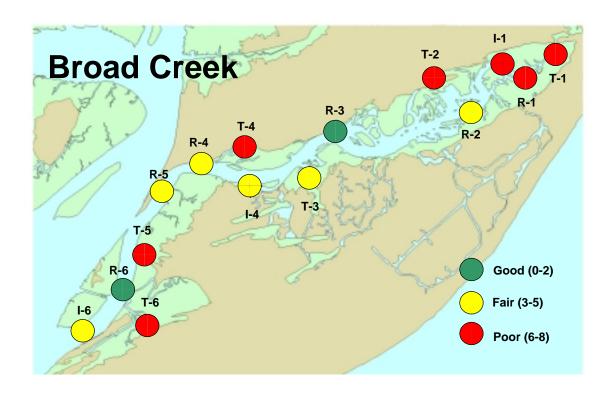


Figure 3.19. Coliform bacteria and multiple antibiotic resistance in Okatee River, indicating relatively low coliform bacterial abundance overall, but with some areas of high coliform counts.



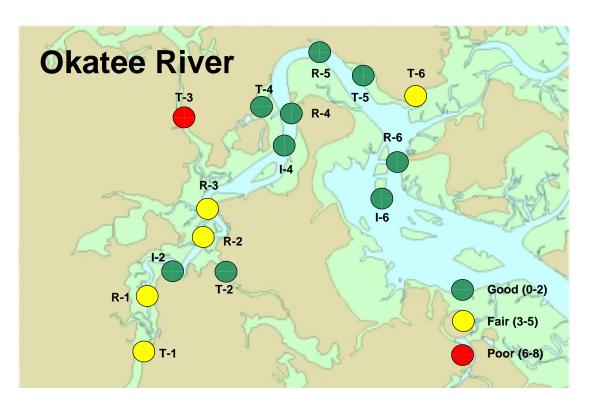


Figure 3.20. Overall water quality summary. See text for scoring explanation.

 Table 3.1.
 Selected water quality results

								Tot.							Fecal	
Station	Sample	Water	Turb.	DO	DO	BOD	PH	Alkal.	Salinity	NH3+NH4-	Total Kelj	NO3-NO2	Total Phos.	TOC	Coliform	Chlor.
	Depth (m)	Temp. °C	NTU	mg/l	% Sat	5-day mg/l	SU	CaCO3 mg/l	ppt	mg/l	N mg/l	mg/l	mg/l	mg/l	/100 ml	a ug/l
Broad Creek	c (8/20/97)															
I-1	0.3	31	32	5.4	72	4.6	7.35	108	25	0.08	0.73	< 0.02	0.19	40	65	31.3
I-4	0.3	31	39	4.3	57.3	2.2	7.4	104	27	0.11	0.72	<0.02	0.17	40	210	17.1
I-6 I-6	0.3 1.1	30.5 30.5	37	5.5 5.6	71.7 73.7	1.8	7.8	107	31 31.5	Lab Error	0.44	<0.02	0.1	58	50	9.8
R-1	0.3	32	45	5.4	73	3.8	7.4	108	24	0.1	0.84	<0.02	0.21	38	80	32.3
R-2 R-2	0.3 2	31.5 31.5	20	6.1 5.8	81.3 77.3	2.4	7.3	108	25.5 25.5	0.56	0.57	<0.02	0.15	42	30	25.1
R-3 R-3	0.3 11	30 30	25	5 5	65.8 65.8	1.6	7.1	107	25 26	Lab Error	0.54	0.02	0.15	38	26	14.1
R-4 R-4	0.3 6	30 30	21	5.8 5.6	75.7 73.7	1.5	8	107	27 27	Lab Error	0.46	0.03	0.11	39	1600	16.5
R-5 R-5	0.3 5	30 30	20	5.9 5.7	77.6 75	8.0	8.1	106	27 28	Lab Error	0.4	0.02	0.08	53	50	13.8
R-6 R-6	0.3 6.5	31 30	23	7.5 7.3	100 95.4	1.8	8.1	106	29 29	Lab Error	0.36	<0.02	0.06	40	110	16
T-1	0.3	29	18	3.3	42.3	2	6.7	68	11	0.31	1	0.34	0.19	27	1600	18.1
T-2	0.3	34	75	6.7	93.7	4	7.45	106	25.5	0.68	1.22	<0.02	0.32	44	900	51.1
T-3 T-3	0.3 1.4	31 31	7	4.5 4.5	59.3 59.3	2.4	7.2	86	16 16	0.14	0.68	0.05	0.35	36	>1600	23
T-4	0.3	28.5	200	2.4	30.4	2	6.45	39	27	0.32	1.6	0.22	0.72	33	>1600	18
T-5	0.3	32	12	4.8	64.9	3.3	7.2	106	28	Lab Error	0.39	<0.02	0.08	46	>1600	9.1
T-6 T-6	0.3 2	29 29.5	8.2	3.9 3.1	50 39.1	2.3	7.1	91	10.5 28	0.27	0.92	0.07	0.68	42	>1600	32.6

Table 3.1. Continued

Station	Sample Depth (m)	Water Temp. °C	Turb.	DO mg/l	DO % Sat	BOD 5-day mg/l	PH SU	Tot. Alkal. CaCO3 mg/l	Salinity	NH3+NH4- mg/l	Total Kelj N mg/l	NO3-NO2	Total Phos.	TOC mg/l	Fecal Coliform /100 ml	Chlor. a ug/l
Okatee Rive			1110	mg/i	70 Out	mg/i		mg/i	ppr	mg/i	9/1	9/.	g,ı	mgn	7100 1111	ugri
I-2	0.3	32	14	4.3	58.1	2.6	7.3	109	28	Lab Error	0.52	<0.02	0.07	5.1	13	17.1
I-2	0.9	31.1	20	4.4	58	2.2	7	110	27	Lob Error	0.50	-0.00	0.12	<i>-</i> 7	12	04
I-4	0.3	32	30	6.1	82.4	2.2	7	110	29	Lab Error	0.59	<0.02	0.13	5.7	13	21
I-6 I-6	0.3 0.95	31 30	7.3	3.1 3.3	40.7 42.8	1.4	7.1	111	31 31.5	0.1	0.36	<0.02	0.09	4.2	<2	6.1
R-1 R-1	0.3 1.2	32.5 32.3	70	5.9 5.8	79.1 78.4	3.4	6.8	104	25.5 25	Lab Error	0.97	<0.02	0.16	8.5	90	38.5
R-2 R-2	0.3 1	32.6 32.2	32	5.5 5.4	75.3 72.7	2.7	7.5	106	27 27	0.74	0.85	<0.02	0.12	5.2	40	30
R-3 R-3	0.3 2	31.5 31.9	45	6 5.1	79.7 68.9	2.6	7.1	108	27 27	0.68	0.97	<0.02	0.1	7.2	13	22.8
R-4 R-4	0.3 4	31 31	31	5.5 5.3	73.3 70.7	1.8	6.9	108	26 27.5	0.42	0.68	<0.02	0.12	6.1	8	15.1
R-5 R-5	0.3 5	30.5 30.5	30	3 2.5	39.5 32.9	1.4	6.9	108	26 28	0.48	0.68	0.02	0.12	5.7	4	8.9
R-6 R-6	0.3 4	30.5 30.5	33	3.5 3.5	46.1 46.1	1.6	6.2	110	30 28	0.46	0.76	<0.02	0.1	5.6	<2	10.5
T-1	0.3	29	45	6.4	81.4	1.6	6.9	25	19.5	0.33	0.88	0.1	0.28	8.4	280	9.1
T-2	0.3	32	40	4.7	62.8	1.4	7.25	108	27	Lab Error	0.82	<0.02	0.16	6.6	23	7
T-3	0.3	30.5	30	6.6	86.3	5.9	7.2	91	18	0.14	1.08	<0.02	0.19	10.3	1600	40
T-4 T-4	0.3 0.7	33.2 31.2	13	5 5.2	68.5 68.7	2.4	7.3	110	28 28	Lab Error	0.53	<0.02	0.1	6.3	30	24.5
T-5	0.3	32	27	5	67.6	1.9	7.4	111	29	Lab Error	0.55	<0.02	0.12	5.9	23	15.1
T-6 T-6	0.3 0.8	31.1 31	16	4.6 4.6	61.3 60.7	1.6	6.4	111	29 29	Lab Error	0.56	<0.02	0.08	4.7	2	12

Table 3.2. Selected Water Quality Summary Statistics from all SCDHEC Saltwater Monitoring Sites 1993-1997

		50th	90th	95th	
Parameters	N	Percentile	Percentile	Percentile	MEAN
Alkalinity (mg/l)	2688	79	110	114	72.4
TOC (mg/l)	1016	6.6	16	25	9.33
BOD_5 (mg/l)	3147	1.4	2.6	3.2	1.66
Turbidity (NTU)	3178	9	25	34	13
NH3+NH4 (mg/l)	2785	0.05	0.11	0.25	0.08
TKN (mg/l)	2845	0.59	1.06	1.26	0.68
NO3+NO2 (mg/l)	3348	0.05	0.2	0.28	0.09
TP (mg/l)	3329	0.06	0.16	0.28	0.09

Source: SCDHEC. 1998b.

Table 3.3. Summary of selected Hydrolab readings

			Disso	lved Oxy	/gen (m	ng/l)			Percent Dissolved Oxygen Saturation				Salinity (ppt)					pH (SU)						
Station	N			Range			%<2	N	Min	Max	Range	Avg	N<28	%<28	N			Range	Avg	N	Min	Max	Range	Avg
BROAD	CRE	EΚ																						
R-1	48	1.63	6.63	5	4.222	4	8.33	48	24.5	105.2	80.7	65.72	3	6.25	48	25.8	28.9	3.1	27.97	48	7.13	7.62	0.49	7.415
R-2	51	2.44	5.97	3.53	4.382	0		51	37.8	94.2	56.4	68.14	0		51	27.6	29.5	1.9	28.58	51	7.12	7.46	0.34	7.348
R-3	50	3.53	5.43	1.9	4.622	0		50	54.6	86.0	31.4	72.51	0		50	26.4	29.7	3.3	27.83	50	7.17	7.59	0.42	7.370
R-4	50	3.63	5.68	2.05	4.627	0		50	56.3	89.8	33.5	72.46	0		50	26.5	30.3	3.8	28.08	50	7.18	7.76	0.58	7.419
R-5	51	3.92	5.51	1.59	4.964	0		51	58.4	85.0	26.6	75.68	0		51	29.7	31.8	2.1	30.82	51	7.53	7.91	0.38	7.734
R-6	51	4.25	5.78	1.53	5.085	0		51	64.5	90.1	25.6	78.57	0		51	30.4	33.2	2.8	31.65	51	7.50	7.93	0.43	7.731
T-1	40	0.72	6.09	5.37	3.628	7	17.50	40	10.5	95.5	85.0	54.91	5	12.50	40	6.4	28.7	22.3	24.01	40	2.50	7.33	4.83	5.317
T-2	38	2.20	6.48	4.28	4.298	0		38	33.6	102.7	69.1	66.52	0		38	26.9	28.9	2.0	28.31	38	7.12	7.66	0.54	7.427
T-3	36	2.43	5.10	2.67	3.389	0		36	35.9	77.5	41.6	52.11	0		36	16.6	26.9	10.3	23.43	36	7.44	7.81	0.37	7.668
T-4	35	1.84	4.29	2.45	3.297	1	2.86	35	28.3	67.6	39.3	51.46	0		35	15.2	28.8	13.6	27.05	35	7.44	7.88	0.44	7.656
T-5	51	80.0	8.08	8	3.923	10	19.61	51	1.1	126.8	125.7	59.80	10	19.61	51	30.4	31.9	1.5	31.34	51	6.97	7.80	0.83	7.373
T-6	51	3.11	6.36	3.25	4.700	0		51	42.4	96.5	54.1	69.28	0		51	4.7	31.8	27.1	25.79	51	7.17	7.87	0.70	7.530
OKATE	E RIV	'ER																						
R-1	51	3.03	7.00	3.97	5.233	0		51	45.4	111.0	65.6	81.24	0		51	24.5	30.1	5.6	28.98	51	7.10	7.51	0.41	7.349
R-2	50	4.05	6.92	2.87	5.225	0		50	62.3	108.8	46.5	81.02	0		50	29.1	30.2	1.1	29.71	50	7.28	7.53	0.25	7.402
R-3	51	3.27	6.08	2.81	4.310	0		51	51.9	99.0	47.1	69.07	0		51	28.1	29.8	1.7	28.95	51	7.29	7.56	0.27	7.395
R-4	51	3.00	5.88	2.88	4.154	0		51	47.5	95.1	47.6	66.23	0		51	26.7	29.6	2.9	28.60	51	7.34	7.57	0.23	7.423
R-5	50	4.65	6.32	1.66	5.405	0		50	69.6	96.5	26.9	81.89	0		50	30.0	31.2	1.2	30.70	50	7.43	7.57	0.14	7.490
R-6	51	4.62	6.51	1.89	5.441	0		51	68.2	99.5	31.3	82.39	0		51	30.8	31.4	0.6	31.10	51	7.37	7.55	0.18	7.449
T-1	42	2.67	7.03	4.36	4.653	0		42	37.4	100.7	63.3	69.49	0		42	2.4	28.8	26.4	22.74	42	6.90	7.45	0.55	7.246
T-2	49	1.02	6.66	5.64	4.616	1	2.04	49	14.7	105.1	90.3	71.66	1	2.04	49	27.0	30.1	3.1	29.56	49	7.16	7.53	0.37	7.346
T-3	36	3.12	6.67	3.55	3.897	0		36	49.4	108.3	58.9	62.18	0		36	28.1	29.3	1.2	28.79	36	7.32	7.67	0.35	7.438
T-4	50	3.55	7.42	3.87	4.619	0		50	46.0	118.2	72.2	70.93	0		50	10.1	28.3	18.2	23.92	50	6.99	7.72	0.73	7.386
T-5	49	3.13	8.95	5.82	5.466	0		49	44.3	135.9	91.6	82.63	0		49	30.2	31.2	1.0	30.82	49	7.19	7.59	0.40	7.404
T-6	44	3.00	8.10	5.1	4.553	0		44	45.6	114.2	68.6	69.89	0		44	9.1	31.6	22.5	30.45	44	7.14	7.59	0.45	7.343

N<2 = Number of dissolved oxygen measurements less than 2.0 mg/l

^{%&}lt;2 = Percentage of dissolved oxygen measurements less than 2.0 mg/l

N<28 = Number of percent dissolved oxygen values less than 28%

^{%&}lt;28 = Percentage of percent dissolved oxygen values less than 28%

Table 3.4. Summary of MAR results from Broad Creek, the Okatee River and selected Sewerage Treatment Plants in coastal South Carolina. Note that *E. coli* bacteria from STPs were more resistant to larger number of antibiotics than was measured in surface water samples from Broad Creek and the Okatee River. In addition, *E. coli* bacteria in Broad Creek were more resistant to a larger number of antibiotics than those measured in the Okatee River.

	Estuar	у Туре	
Antibiotic	Undeveloped (Okatee River) n=1061	Developed (Broad Creek) n=1443	Sewage Treatment Plants n=860
Ampicillin	0	4 (0.3%)	18 (2%)
Chlortetracycline	0	9 (0.6%)	6 (0.7%)
Kanamycin	0	1 (0.07%)	0
Naladixic Acid	0	1 (0.07%)	1 (0.1%)
Neomycin	0	0	0
Oxytetracycline	0	16 (1%)	9 (1%)
Penicillin G	10 (0.9%)	6 (0.4%)	46 (5%)
Streptomycin	0	0	8 (0.9%)
Sulfathiazole	1 (0.09%)	0	6 (0.7%)
Tetracycline	0	12 (0.8%)	12 (1%)
Number of Sensitive Treatments	1050 (99%)	1394 (97%)	754 (88%)
Number of Resistant Treatments	11 (1%)	49 (3%)	106 (12%)
Overall MAR (%)	1.04	3.40	12.33

Table 3.5. Regional comparisons of site MARs at developed and undeveloped watersheds in South Carolina (this study), Florida (Parveen et al., 1997) and Maryland (Kaspar et al.,1990). Note that the percentage MAR in developed and undeveloped watersheds were quite similar (47-69%), despite these regional differences.

	SITE I	MAR (%)		
Watershed	Developed	Undeveloped	% Difference (Dev. vs. Undev.)	Reference
Florida (Appalachicola Bay)	25	13	47	Parveen et al., 1997
Maryland (Anacostia R., Anapolis Harbor, Balitimore Harbor vs. Chester R., Miles R., Wye R., and Love Point)	9	2.8	69	Kaspar et al., 1990
South Carolina (Broad Creek vs. Okatee R.)	3.4	1.04	69	This Study

Table 3.6. MAR results for selected Sewerage Treatment Plants in coastal South Carolina. Note the higher MAR Index and the number of antibiotics to which E. Coli strains were resistant in those STPs servicing retirement communities at Hilton Head (LC-1, BC-1 and HH-1) when compared to those serving more general populations of Beaufort County (OK-1, SI-1 and WX-1).

Antibiotic	FTSTP FIN. 12/97 (n=2)	BC-1 (n=13)	HH-1 (n=15)	LC-1 (n=15)	OK-1 (n=15)	SI-1 (n=15)	WX-1 (n=13)
Ampicillin	0	3	3	2	9	1	0
Chlortetracycline	1	3	2	1	0	0	0
Kanamycin	0	0	0	0	0	0	0
Naladixic Acid	0	0	0	1	0	0	0
Neomycin	0	0	0	0	0	0	0
Oxytetracycline	1	4	4	1	9	3	0
Penicillin G	0	10	7	8	0	0	9
Streptomycin	1	3	4	1	0	0	0
Sulfathiazole	0	2	3	1	2	3	0
Tetracycline	1	3	3	1	0	0	0
Total # Resistance	4	28	26	16	20	7	9
Percent Resistant	20%	22%	17%	11%	13%	5%	7%
# Antibiotics Resistant	4	7	7	8	3	3	1

Table 3.7. Threshold values used to determine overall water quality summary value.

Parameter	Threshold
Dissolved Oxygen ¹	24-Hour Average < 5.0 mg/l
pH ¹	<6.5 SU or >8.5 SU
Fecal Coliform Bacteria ¹	>43 colonies/100 ml
Salinity ²	24-Hour Range >20 ppt
Chlorophyll-a ³	>20 ug/l
Alkalinity ⁴	110 mg/l
Total Organic Carbon ⁴	16 mg/l
Five-Day Biochemical Oxygen Demand ⁴	2.6 mg/l
Turbidity ⁴	25 NTU
Total Kjeldahl Nitrogen ⁴	1.06 mg/l
Nitrate/Nitrite Nitrogen ⁴	0.2 mg/l
Total Phosphorus ⁴	0.16 mg/l

¹State Standard (SCDHEC, 1998a)

²Holland et al. 1996

³NOAA (1996)

⁴Statewide 90th Percentile (SCDHEC, 1998b)